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GASTRORETENTIVE FLOATING CUBOSOMAL INSITU GELS OF BALOFLOXACIN

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Abstract

Aim: The objective of this study was to develop novel floating Cubosomal in situ gels of Balofloxacin and evaluate its efficacy in the gastric environment.

Methods: Cubosomes of Balofloxacin were prepared by top down approach by employing Glyceryl mono oleate (GMO) and poloxamer. The prepared Cubosomal dispersions were characterized by encapsulation efficiency, in vitro drug release, particle size and Zeta potential. The optimized Cubosomal dispersion(F4) was used for the formulation of Balofloxacin in situ gels by using sodium alginate alone and in combination with other polymers such as HPMC, Pectin and Carbopol. The prepared in situ gels were characterized for appearance, viscosity, drug content, in vitro drug release and in vivo drug release.

Results: The size of the Cubosomal dispersions were found to be in the range of 100nm to 300nm. Zeta potential value indicates that the Cubosomal dispersions were stable. XRD studies inferred that the Balofloxacin in the Cubosomal gel formulation was less crystalline than the pure Balofloxacin. Less crystalline nature was attributed to the enhancement of solubility and bioavailability. All the formulations exhibited the drug release in the range of 80 to 90 %. Based on 86.4±0.23 % drug release, 5724cps viscosity(firmness), floating ability(>12h) and inherent bioadhesive property, formulation CG 10 was selected for in vivo studies.

Conclusion: The results of in vivo studies showed significant absorption in the gastric environment and bioavailability hence the objective is met.

Key words:

In situ gel, Cubosomes, GMO, Sodium alginate, Pectin, XRD studies.

Introduction

Over the last three decades, various approaches have been pursued to increase the retention of an oral dosage form in the stomach including floating drug delivery systems (FDDS), swelling and expanding systems, bioadhesive systems, modified shape systems, high-density systems and other delayed gastric emptying devices[1]. FDDS are widely explored for gastroretention purposes and have a bulk density lower than gastric fluids and thus remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time[2].

The bioadhesive polymer Glyceryl monooleate(GMO) is a polar amphiphilic lipid when placed in water can be organized intolipid bilayers,forminga reversed micellar phase and three types of liquid crystalline phases (lamellar,reversed hexagonal and the cubic phase).The liquid crystalline phase appears in cubic shape are known as cubosome.

Cubosomes are nanoparticles, more accurately nanostructured liquid crystalline particles[3,4], in a liquid crystalline phase with cubic crystallographic symmetry formed by self-assembly of amphiphilic or surfactant-like molecules. This unique liquid crystalline structure of cubosomes could provide protection for the entrapped drug from degradation in the gastrointestinal tract[5,6]. Indeed, the dispersed cubosomes have been highly recommended as carriers for active molecules due to their low viscosity, large interfacial areas, and the presence of both hydrophilic and hydrophobic regions. Furthermore, because of their great biocompatibility and bioadhesivity, cubosomes are suitable for oral administration[7].

In situ forming polymeric formulations are in sol form before administration in the body, but once administered, undergoes gelation in situ to form gel. The formation of the gel depends upon factors like temperature modulation, pH changes, presence of ions and ultra-violet irradiation. On contact with gastric fluid a gel forming solution (eg; the sodium alginate solution containing carbonates or bicarbonates) swells and forms a viscous cohesive gel containing entrapped carbon dioxide bubbles. This forms a gel and float on the top of gastric fluid which releases the drug slowly in the stomach[9,10].

Balofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Balofloxacin in conventional oral dosage form may not be absorbed completely due to its shorter gastric residence time of 2 hours. Balofloxacin is poorly soluble in water and has absorption window is upper gastrointestinal tract.

Balofloxacin is encapsulated in cubosomes to improve the solubility,stability from gastric enzymes and to control the drug release.The drug release is further controlled by formulating in the form of floating insitu gels to increase gastric residence time there by enhances the bioavailability since the absorption window of Balofloxacin is upper gastrointestinal tract.

A novel strategy by combining both cubosomes and floating insitu gels to develop a sustained release formulation termed as Floating cubosomal in situ gels to prolong the gastric residence time of Balofloxacin upto 12 hours in comparision to conventional dosage form. The unique properties of GMO like cubosomal formation and Sodium alginate form a gel in the presence of ions makes an ideal components suitable for designing gastroretentive drug delivery system.

The aim of the present study was to develop a Floating Cubosomal Insitu Gels of Balofloxacin for gastric specific drug delivery and to evaluate invitro and invivo properties of the developed dosage form.

Materials & Methods

Balofloxacin was a kind gift from Cirex Pharmaceuticals Ltd, Glycerol monooleate was a gift sample from Mohini Organics Ltd, Poloxamer 407 was obtained from the S D fine chem. Ltd, Sodium alginate and Carbopol 934 were gifted from Loba Chemie Pvt Ltd, Hydroxy propyl methyl cellulose was obtained from Colorcon Asia Pvt Ltd. and pectin from Himedia laboratories Pvt Ltd.

Preparation of Balofloxacin Cubosomes:

Varying concentrations of Glyceryl monooleate (GMO) 5 to 50% was heated along with Poloxamer 407 (1 % weight corresponding to GMO conc.) on a water bath at a temperature of 40 to 45^{o}C until Poloxamer 407 completely dissolves in GMO. To the above solution Balofloxacin (100 mg) was added and mixed well. The clear lipid solution obtained was added drop wise to distilled water and subjected to bath sonication upto 45 minutes with intermittent stirring. A white opaque dispersion was obtained without aggregates. The formulations are listed in Table 1.

Table-1: Composition of Cubosomal dispersions.

Formulation code	**Monooleine (%W/V)**	**Poloxamer 407 (%W/W)**	**Balofloxacin (mg)**	**Water (Up to 30 ml)**
F1	10	1	300	q.s
F2	12.5	1	300	q.s

F3	15	1	300	q.s
F4	17.5	1	300	q.s
F5	20	1	300	q.s
F6	20.5	1	300	q.s

Sample size=60ml

Evaluation of Cubosomes:

Particle size analysis:

The size and nature of cubosomes were observed under an optical microscope using a calibrated eyepiece micrometer, and photographs were taken at 400 × magnifications with a digital camera (Sony, 8.1 megapixel, Japan). The diameter of sonicated cubosomes was determined by dynamic light scattering technique using a particle size analyzer (HORIBA SZ-100).

Zeta potential:

Zeta potential was determined using the Zetasizer (HORIBA SZ -100). Measurements were performed on the same samples prepared for size analysis. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

XRD-Analysis:

XRD Analysis were performed for pure drug and formulation using x-ray diffractometer (SHIMADZU XRD-7000).The samples were irradiated with Cu radiation and analyzed between10 and $80^0 2\theta$,voltage and current used were 40 kv and 30 mA respectively. XRD studies were conducted for determining crystallinity of the drug.

Entrapment Efficiency (EE):

Entrapment efficiency is defined as the percentage amount of drug which is entrapped by the cubosomes. For the determination of entrapment efficiency, the un-entrapped drug was first separated by centrifugation at 15000 RPM for 30 minutes. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV visible spectrophotometer at 294 nm. The percent of encapsulation efficiency (EE %) was determined by the following equation:

$$\mathbf{EE\%} = \frac{\mathbf{[Total\ drug] - [free\ drug]}}{\mathbf{Total\ drug}} \times \mathbf{100}$$

In vitro drug release studies:

The diffusion cell consists of a hollow glass cylinder (length 14.6 cm and internal diameter 2.5 cm) made up of borosil glass. One end of the cylinder was covered with Himedia dialysis membrane (cutoff molecular weight: 12000-14000), which was previously soaked in warm water. The diffusion cell was placed in a 500 ml borosil beaker that served as the receptor cell. The receptor cell contained a magnetic bead and was rotated at constant speed. The temperature in the diffusion and receptor cells was maintained at 37^{o}C, with the help of a thermostat. Freshly prepared pH 1.2 buffer placed in the receptor cell. A volume equivalent to 50 mg of drug of each formulation was transferred to the diffusion cell. One milliliter sample was withdrawn from the receptor cell at specified time intervals of 1, 2, 3, 4, 5 and 6 hours. Each time immediately after the removal of the sample, the medium was compensated with fresh buffer (pH 1.2). The samples were analyzed for drug content using a UV spectrophotometer at 294 nm.

Preparation of Balofloxacin In Situ Gels:

Specified quantities of Balofloxacin, sodium alginate, sodium citrate, sodium bicarbonate, and different retardant polymers such as HPMCK15M, Pectin and carbopol were weighed according to the formula given in Table 2 & 3 .Sodium alginate solution was heated to 70^{o}C with stirring. After cooling below 40^{o}C. Optimized Balofloxacin cubosomal suspensions (F4) and other ingredients were added and the volume was made up with water. The prepared formulations were stored in air tight container[10].

Table-2: Composition of Balofloxacin Cubosomal In Situ gels using HPMC and Sodium alginate.

Composition	**Formulations**					
	CG1	**CG2**	**CG3**	**CG4**	**CG5**	**CG6**
Balofloxacin (mg)	300	300	300	300	300	300
Sod. Alginate (%)	1	2	3	3	3	3
HPMC K15M(%)	-	-	-	0.5	0.75	1
Sod.citrate(%)	0.25	0.25	0.25	0.25	0.25	0.25
$NaHCO_{3(\%)}$	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben (%)	0.02	0.02	0.02	0.02	0.02	0.02
WaterUpto30ml	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Sample size=60ml

Table-3: Composition of Balofloxacin Cubosomal In Situ gels using pectin and Carbopol.

Composition	Formulations					
	CG7	**CG8**	**CG9**	**CG10**	**CG11**	**CG12**
Balofloxacin (mg)	**300**	**300**	**300**	**300**	**300**	**300**
Sod. Alginate (%)	3	3	3	3	3	3
Pectin(%)	0.5	0.75	1	-	-	-
Carbopol (%)	-	-	-	0.5	0.75	1
Sod. citrate (%)	0.25	0.25	0.25	0.25	0.25	0.25
$NaHCO_{3(\%)}$	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben (%)	0.02	0.02	0.02	0.02	0.02	0,02
WaterUpto30ml	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Sample size=60ml

Evaluation of Floating Cubosomal in-situ gels of Balofloxacin:

Physical appearance

The appearance was checked visually. The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

Surface morphology

The morphology of cubosomes was determined using scanning electron microscopy (Hitachi S-3700N). SEM gives a three dimensional image of the globules. The samples were examined at suitable accelerating voltage 20 KV, at different magnifications. A good analysis of surface morphology of the disperse phase in the formulation can be obtained through SEM.

Determination of Drug Content

Accurately, 10 ml of *in-situ* gel (equivalent to 100 mg of Balofloxacin) was measured and transferred to 100 ml of volumetric flask. To this 50-70 ml of 0.1 N HCl was added and shaken on a mechanical shaker for 30 min, followed by sonication for 15 min complete dispersion of contents were ensured, visually and filtered using 0.45 µ membrane filter From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1 N HCl. Content of Balofloxacin was determined spectrophotometerically at 294 nm using double beam UV-Visible spectrophotometer.

Determination of Viscosity

The viscosity of the samples was determined using Brookfield Digital Viscometer (Model DV Pro). The formulation (100 ml) was taken in a beaker and maintained at room temperature. Viscosities were determined at different shear rates from 00 to 100 rpm at room temperature.

In-vitro release studies

The drug release studies were carried out in USP XXVI dissolution test apparatus using basket apparatus at 37 ± 0.5°C at 50 rpm using 900 ml of pH 1.2 buffer as a dissolution medium (n=6). *In-situ* gel equivalent to 100 mg of Balofloxacin (10 ml) was used for testing. 5 ml of aliquot was withdrawn at predetermined time interval. The contents were filtered using 0.45 µ nylon filters and analyzed at 294 nm spectrophotmetrically. Same volume of dissolution fluid maintained at 37 ± 0.5°C was replaced immediately.

In-Vivo release studies

Male Wister rats weighing (100-120gm) were fasted overnight before experimentation and had free access to water. The protocol and procedures were approved by the animal ethics committee reference number 1722/RO/Ere/S/13/CPCSEA.The rats were divided into two groups of three rats each. Balofloxacin pure drug and optimized cubosomal in situ gel were administered orally for the two groups respectively. The dose administered was 20mg /kg of animal weight.

Blood samples were withdrawn from the retro orbital plexus at an interval of 0,2,4,6,8,10, and12 hrs. Then the blood samples were subjected to centrifugation to separate plasma. The concentration of Balofloxacin in plasma was determined by HPLC method. HPLC analysis was performed by using column-Inertsil, the mobile phase is methanol: acetonitrile: phosphate buffer pH 5.4 (50: 20: 30) at a flow rate of 1ml/min, injection of the sample was 20µl and detected at λ_{max} 298 nm[8].

Results and Discussion

The prepared cubosomes (Fig 1) were evaluated for various parameters such as Particle size distribution by the Horiba particle size analyzer, surface morphology by Scanning electronic microscopy, Zeta potential, drug entrapment efficiency, in vitro and in vivo drug release studies.

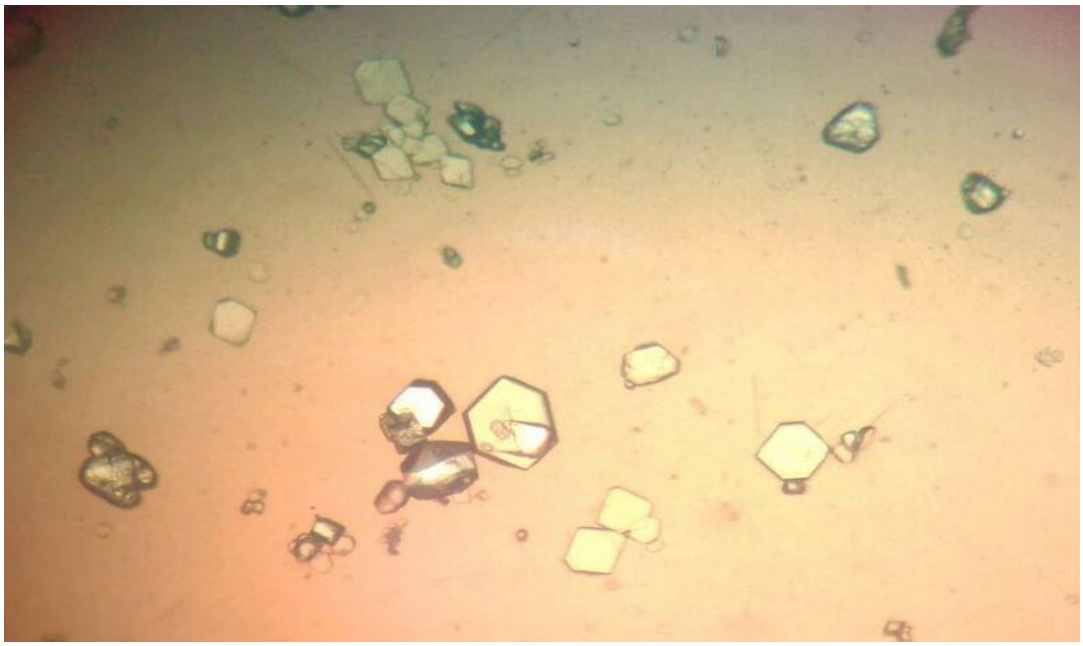


Fig. 1: Photomicrograph of Cubosomes.

Particle size analysis:

The particle size of cubosomes was determined by the Horiba particle size analyzer.

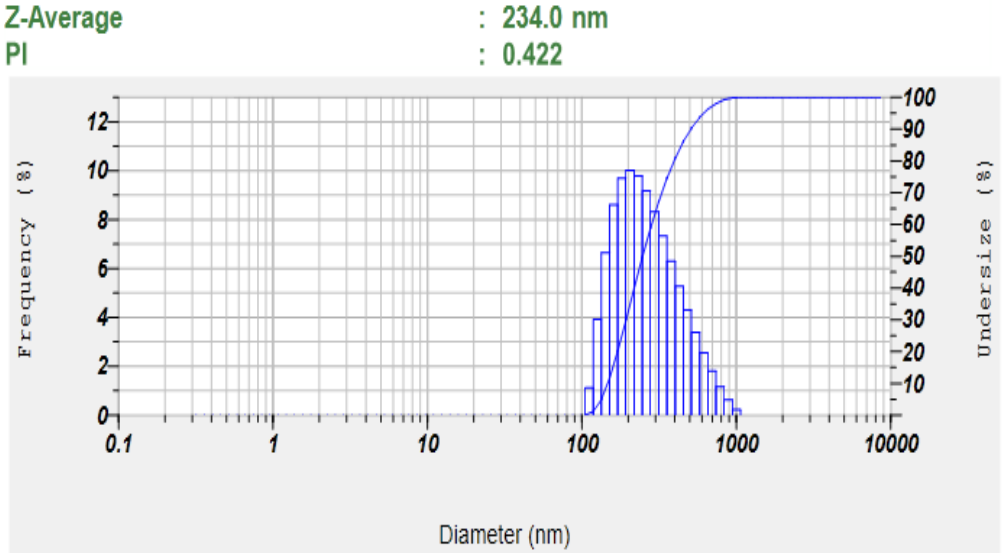


Fig 2: Particle size analysis of Cubosomes

From the Figure 2 it was found that the diameter (nm) of cubosomes was found to be in the range of 100 to 300 mm and the average particle size was found to be 234.0 mm.

Zeta Potential:

The zeta potential of cubosomes was determined using Zeta sizer and value was found to be -9mv as shown in the figure 3 which indicates that cubosomes were stable.

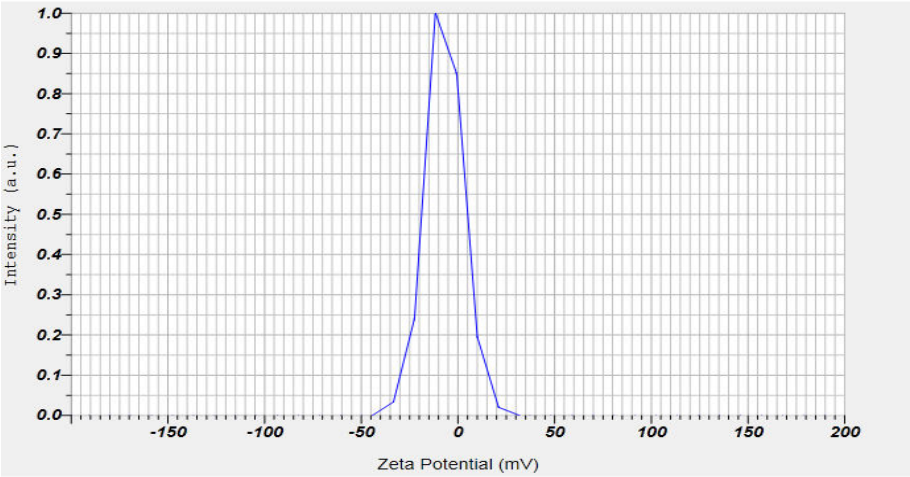


Fig 3: Zeta potential of Cubosomes.

X-ray Diffraction Studies:

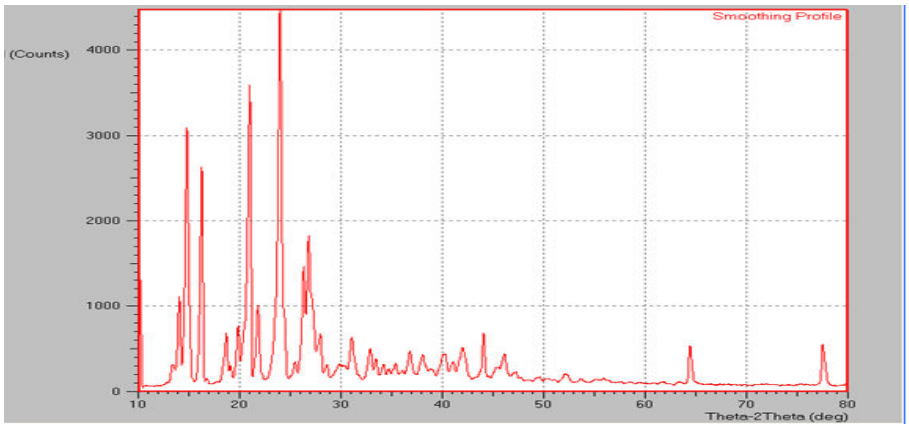


Fig 4: Scan of X-ray diffraction of Plain drug.

Fig 5: Scan of X-ray diffraction of Cubosomal gels.

Table 4: Results of X-ray diffraction studies.

Material	**2-Theta**	**Intensity**
Plain drug	31	121
Cubosomal formulation	31	6

X-ray diffraction studies (Figure 4, 5 and Table 4) inferred that the Balofloxacin in the Cubosomal gel formulation was less crystalline than the pure Balofloxacin. Less crystalline nature was attributed to the enhancement of solubility and bioavailability

Drug Entrapment efficiency: Drug entrapment efficiency of Cubosomal dispersions was shown in figure 6.

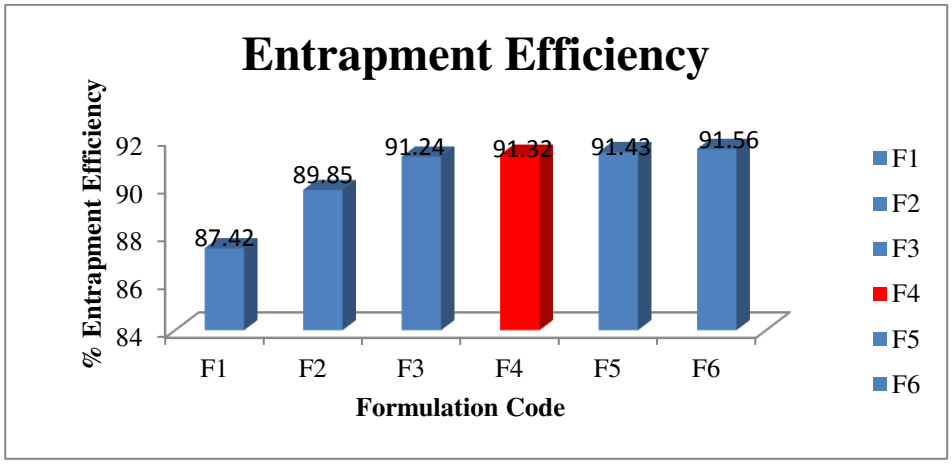


Fig 6: Entrapment efficiency of Cubosomal dispersions.

From the above figure the Entrapment efficiency was found to increase from 87.42 to 91.56% (w/w) upto Formulation F6 by increasing GMO concentration from 10 to 20%. This was due to the partitioning of the drug into the GMO phase.Hence it is inferred that GMO was able to form cubic phases at a particular concentration of water (i.e. at 17.5 % GMO and 82.5 % water).Above 17.5% of GMO there was no diiference in entrapment efficiency and also phase separation observed in F5 and F6. So Formulation F4 was selected for further studies based on high entrapment(91.32 %) and stability.

Drug Release Studies: The drug release values of the cubosomal dispersions are shown below:

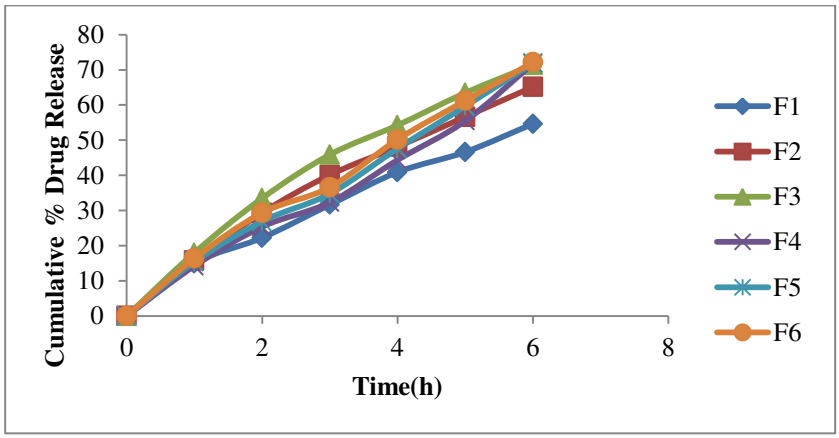


Fig 7: Invitro drug release profile of Cubosomal dispersions.

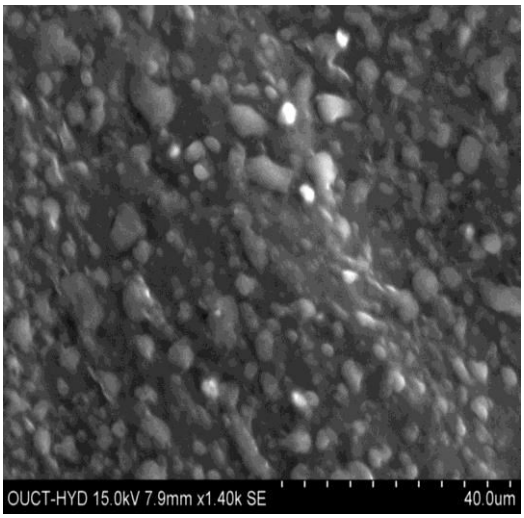
Figure 7 presents the drug release data expressed on percentage basis. The fraction of Balofloxacin released as a function of time from cubosomal dispersions from F1 to F6 was 15 to 16.36 % at the end of first hour indicating that the drug release was independent of initial drug loading.The solubility and concentration of the incorporated drug influences the release profile from cubsomal dispersions[11]. Location of the drug is an important parameter affecting release and kinetics. For example lipophilic drugs become incorporated into lipid bilayers and thus partitioning into the aqueous phase becomes the rate limiting step. Therefore, the effect of drug loading on the drug release profile depends on the drug partioning between the GMO and aqueous phase[12]. Formulation F4 was selected for gel preparation on the basis of highest drug release71.80%.

Evaluation of Cubosomal Insitu gel

The prepared cubosomal gels were evaluated for various parameters such as homogenicity, pH, viscosity, in vitro drug release studies and invivo drug release studies.

Homogeneity: It was evaluated by visual observations(Figure 8). All formulations were found to be homogenous and opaque.

Surface Morphology: The surface morphology of cubosomes in gel was determined by SEM analysys and optical microscopy.



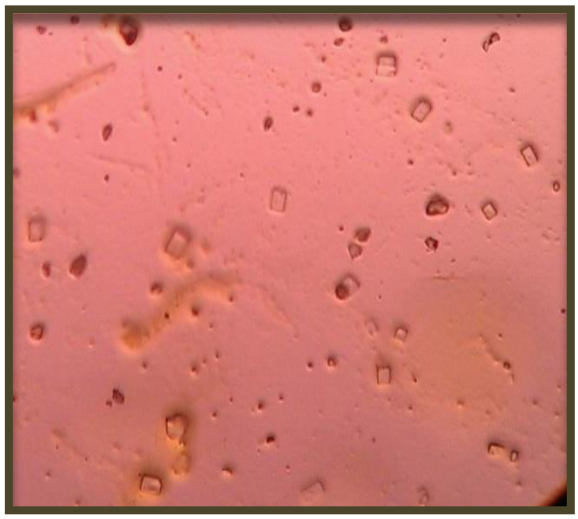


Fig 8:A)SEM image of Cubosomal gel. B) Optical micro photograph of Cubosomal gel.

It was inferred from SEM image and Microscopy that cobosomes are clearly seen in floating cubosomal in situ gels.

Drug Content:

The drug content of all formulations were shown in table 5.

Table-5: Drug content of various formulations of Balofloxacin Cubosomal Gels.

Formulation code	Drug content%*
CG1	96.7±0.64
CG2	94.6±0.65
CG3	92.4±0.51
CG4	91±0.87
CG5	89.2±0.13
CG6	87.1±0.65
CG7	92.2±0.87
CG8	91±0.32
CG9	89.6±0.96
CG10	93±0.68
CG11	92.3±0.74
CG12	89±0.54

*All values are in mean±SD(n=3)

It was observed from the above table that the drug content of all the formulations was found to be in the range of 85-98%.

Determination of viscosity:

The viscosity of all the formulations were determined by Brooke field viscometer.

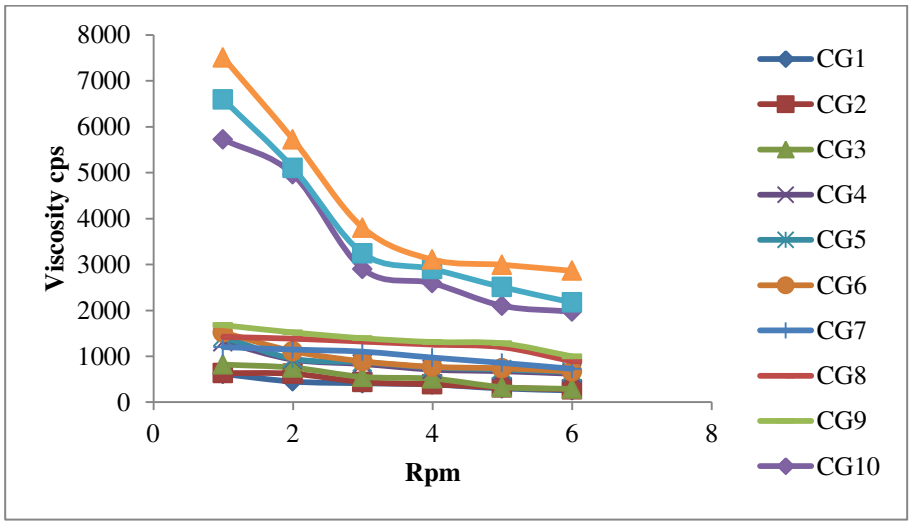


Fig 9: Viscosity of Cubosomal gels

It was inferred from the above study (Figure 9) that the formulations containing carbopol(CG10 to CG12) exhibits greater viscosity than formulations containing sodium alginate alone and with combination with HPMC and pectin(CG1 to CG9).Formulation CG10 exhibits pseudoplastic property compared to other formulations.

Invitro release profile of cubosomal gels:

The invitro release profiles of cubosomal gel formulations are shown in figure 10 and 11.

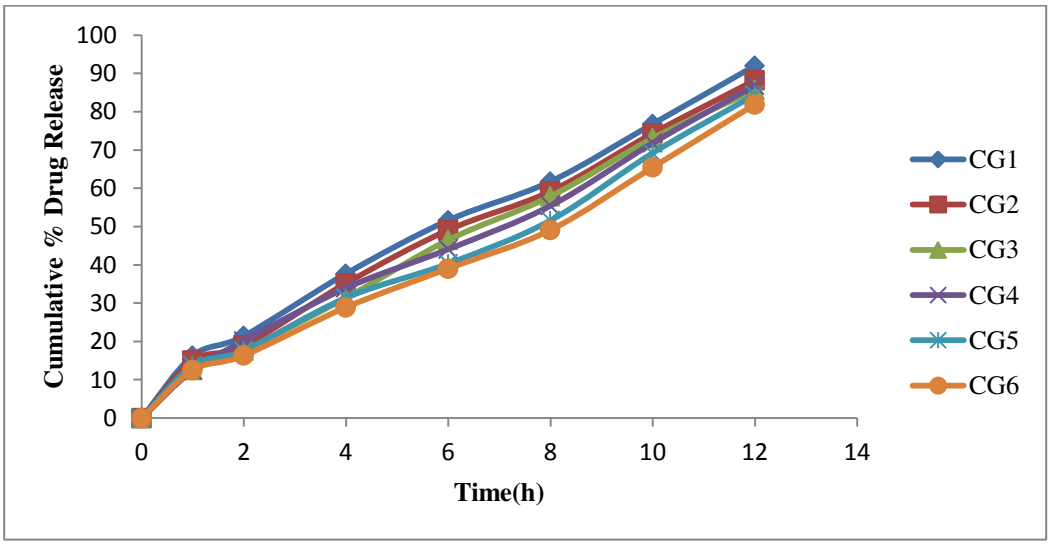


Fig 10: Invitro release profile of Cubosomal gels

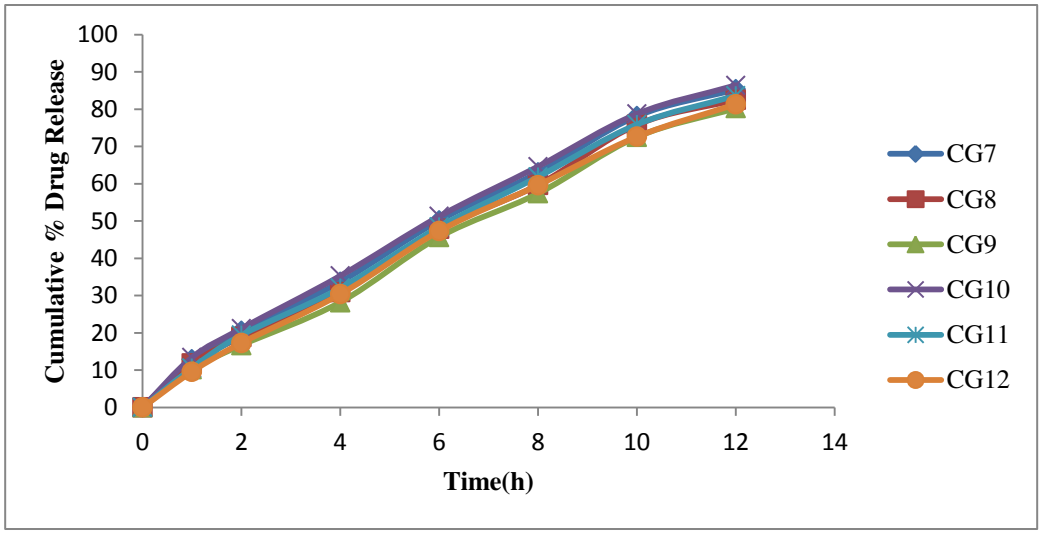


Fig 11: Invitro release profile of Cubosomal gels

At the end of 12 hours, formulations containing sodium alginate(CG1 to CG3) alone exhibited a drug release of 85 to 92 % and those formulations containing sodium alginate with HPMC(CG4 to CG6) exhibited a drug release of 81 % to 86 % whereas formulations containing sodium alginate with pectin(CG7 to CG9)exhibited a drug release of 80 % to 85 % and formulations containing sodium alginate with carbopol(CG10 to CG12) had shown the drug release of 81 % to 86 %. Formulations with HPMC and pectin showed highest drug release but the firmness(high viscosity) and floating ability was observed with formulations containing combination of sodium alginate with carbopol. Hence on the basis of drug content(93 %), drug release(86.4%) and viscosity(5724 cps), formulation CG10 was selected for invivo studies.

In vivo drug release:

The results of in vivo studies i.e. the pharmacokinetic parameters C_{max} , t_{max} and AUC for both raw Balofloxacin and formulation CG10 were listed in table 6 and fig 12.

Table 6: The results of in vivo drug release studies.

Material	C_{max} (ng/ml)	t_{max} (hrs)	AUC (ng hr/ml)
Puredrug (raw Balofloxacin)	99.96 ± 0.35	2 ± 0.28	497.39 ± 3.52
CG10	100.06 ± 0.58	4 ± 0.21	693.025 ± 3.89

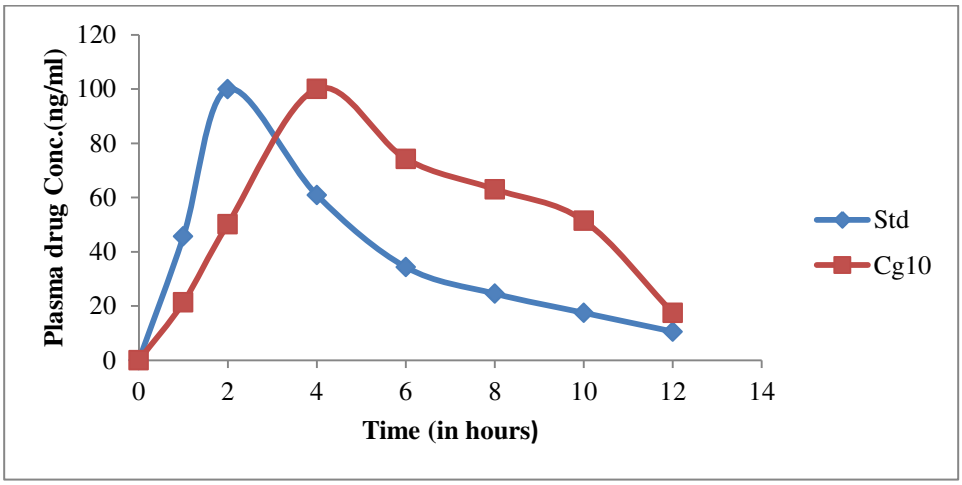


Fig.12: Plasma Drug Concentration time profile of Balofloxacin Formulation and pure drug

From the above results it was observed that the $C_{max}$and t_{max} for both pure drug(raw Balofloxacin) and formulation were 2 hrs and 4 hrs respectively. The AUC values observed for pure drug and formulation were 497.39 ng.hr/ml and 693.025 ng.hr/ml. The results indicates that the bioavailability of floating cubosomal in situ gels was more than the suspension of raw balofloxacin Hence the above findings are a convincing evidence that the floating cubosomal insitu gels effectively sustain the drug release compared to raw Balofloxacin suspension.

Conclusion: Based on the results obtained, it can be concluded that the formulation CG10 was found to be an ideal formulation considering its drug content, drug release and floating ability The value of Zeta potential indicates the formulation was stable. From the results of XRD studies and in vivo studies, it was concluded that the drug is in less crystalline form and absorbed well in the stomach leads to enhancement of bioavailability, hence the objective was met. Thus the optimized formulation was stable, reproducible and ideal for the gastroretentive drug delivery.

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